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Binding of platelet factor four (PF 4) to glomerular polyanion

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Binding of platelet factor four (PF 4) to glomerular polyanion. Synthetic polycations have been shown to bind to glomerular polyanion (GPA) and increase glomerular permeability. Here, we show that human platelet factor 4 (PF 4), a platelet secretory protein, binds to GPA. The following methods were used to assess PF 4 binding to GPA: *(1)* Sections of human and rat renal cortex were incubated with PF 4 or PF 4 was injected intravenously into rats followed by immunofluorescence techniques; *(2)* ^{125}I-PF 4 was added to isolated glomerular basement membrane (GBM) suspensions and binding assessed isotopically; *(3)* PF 4 was perfused through rat kidneys ex vivo followed by immunoperoxidase methods for electron microscopy (EM). In vitro and in vivo, PF 4 bound to the mesangium and linearly to capillary walls. Isotopic studies showed dose-dependent saturable binding of PF 4 to GBM which was reversed by heparin. By EM, PF 4 binding sites were resolved in the GBM, particularly in the laminae rarae as punctate densities similar in distribution to anionic sites revealed by cationic dyes. Also, endothelial and epithelial cell surfaces stained. An ionic interaction between PF 4 and GPA was indicated by elimination of staining by washing PF 4-treated sections with buffer containing 1.0 and 3.0 M NaCl or with heparin. Pretreatment of rats with polyethyleneimine (a synthetic polycation) blocked PF 4 binding. Heparin administration in vivo removed previously bound PF 4. By virtue of its affinity for GPA and behavior like a polycation, PF 4 may alter glomerular permeability and play a role in glomerular disease.

Liaison du facteur plaquettaire quatre (PF 4) aux polyanions glomérulaires. Il a été démontré que des polycations synthétiques se lient aux polyanions glomérulaires (GPA) et augmentent la perméabilité glomérulaire. Ici, nous montrons que le facteur plaquettaire 4 (PF 4), une protéine sécrétoire plaquettaire, se lie au GPA. Les méthodes suivantes ont été utilisées pour préciser la liaison du PF 4 au GPA: *(1)* Des sections de cortex de reins d'homme et de rat ont été incubées avec du PF 4, ou le PF 4 a été injecté par voie intraveineuse à des rats, avec ensuite techniques d'immunofluorescence; *(2)* ^{125}I-PF 4 a été ajouté à des suspensions de membranes basales glomérulaires isolées (GBM) et la liaison mesurée de façon isotopique; *(3)* le PF 4 a été perfusé à des reins de rat ex-vivo avec ensuite technique d'immunoperoxidase pour microscopie électronique (EM). In vitro et in vivo, le PF 4 se liait au mésangium, et de façon linéaire aux parois capillaires. Des études isotopiques ont montré une liaison saturable, dose dépendante, du PF 4 aux GBM, qui était réversible par de l'héparine. En EM, les sites de liaison du PF 4 étaient visibles sur la GBM, surtout sur les laminae rarae, sous forme de densifications ponctuées, identiques aux sites de distribution anionique révélés par les colorants cationiques. Egalement, les surfaces cellulaires endothéliales et épithéliales étaient colorées. Une interaction ionique entre PF 4 et GPA était indiquée par l'élimination de la coloration en lavant des sections traitées au PF 4 avec un tampon contenant 1,0 ou 3,0 M NaCl, ou avec de l'héparine. Le prétraitement de rats avec du polyéthylènimine (un polycation synthétique) a bloqué la liaison du PF 4. L'administration d'héparine in-vivo a enlevé le PF 4 préalablement lié. En raison de son affinité pour le PGA, et de son comportement comme un polycation, PF 4 pourrait altérer la perméabilité glomérulaire et jouer un rôle dans les glomérulopathies.

The glomerular basement membrane (GBM) contains a number of polyanionic substances which impart an overall electronegative charge to the structure, forming a charge barrier to circulating anionic molecules. Recent studies indicate that circulating exogenous synthetic polycations can bind to polyanion within the GBM [1–4]. Such binding to glomerular polyanion has been shown to neutralize the electrostatic barrier leading to alterations in glomerular permeability to circulating anionic macromolecules [1, 4–6]. Preliminary studies from this laboratory have also shown that a synthetic polycation, polyethyleneimine (PEI) can increase glomerular permeability and lead to enhanced deposition of immune complexes [7]. This concept is of considerable clinical importance since a number of inflammatory cells and platelets contain a variety of secretory cationic proteins, which upon release during disease processes, might potentiate immune complex deposition and glomerular injury at a local level. Indeed, platelets have been implicated as mediators in glomerulonephritis [8–11].

If endogenous cationic proteins were to influence glomerular permeability, obviously they must first interact with glomerular polyanion (GPA). The objective of the present study was to examine the potential of a platelet-derived secretory protein, platelet factor 4 (PF 4), which behaves like a polycation, to bind to GPA in the glomerular capillary wall, particularly to the GBM.

Methods

Platelet factor 4 (PF 4). Human PF 4 was purified as previously described [12]. Briefly, outdated platelet concentrates, generously supplied by Wadley Institutes of Molecular Medicine, Dallas, Texas, were washed in citrate saline buffer, then lysed by repeated freeze-thawing. Following centrifugation of membranes, the PF 4-enriched supernate was passed through a heparin-ε-aminocaproic acid-Sepharose column, and washed extensively with buffer. Purified PF 4 was eluted from the column utilizing a gradient of 0.5 to 3.0 M NaCl in 0.005 M sodium barbital buffer, pH 7.4. Purity and activity of PF 4 fractions were assayed according to the methods outlined previously [12]. Antiserum against purified PF 4 was generated and harvested from New Zealand white rabbits by standard laboratory techniques and was shown to form one line of identity with the purified antigen in immunodiffusion plates.

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PF 4 binding to kidney cortical tissue. Binding of PF 4 to human and rat kidney cortex was assessed by indirect immunofluorescence according to the following procedure: PF 4 (590 to 720 μg/ml) in 0.6 M NaCl, 0.005 M barbital buffer, pH 7.4 was diluted 1:4 with 1.0% bovine serum albumin in water. Human kidney cortex was acquired as surplus biopsy or autopsy tissue from the Department of Pathology, The University of Texas Health Science Center at San Antonio or from the South Texas Organ Bank Inc., San Antonio, Texas. Only tissue samples determined to be disease-free by pathological examination were used in these experiments. Rat kidneys were washed free of blood by perfusion with 0.02 M phopshate buffered saline, pH 7.4 (PBS). The kidney cortical tissue was trimmed and then frozen with Cryokwik™ liquid freon or in isopentane pre-chilled in liquid nitrogen. Frozen sections 6 to 8 μm thick were cut using a cryostat (Model CTI, International Equipment Company, Needham Heights, Massachusetts). PF 4 was then layered onto the tissue section and allowed to incubate for 15 min, washed with barbital buffered saline containing 0.15 M NaCl in 0.005 M barbital, pH 7.4 (BBS), then followed by incubation with rabbit anti-human PF 4 serum. Following three additional washes with BBS, the sections were incubated with fluorescein-conjugated IgG fraction of goat anti-rabbit IgG (Cappel Laboratories, Westchester, Pennsylvania) followed again by three washes of BBS and then mounted. Controls consisted of layering diluent without PF 4 in place of the PF 4 solution and/or normal non-immune rabbit serum in place of the primary anti-PF 4 serum. Sections were examined using a Zeiss Universal II Research microscope equipped for epifluorescence using a standard FITC filter set supplemented with a KP560 short-wave pass filter for optimal illumination for fluorescein.

To examine an ionic interaction between PF 4 and GPA, sections were washed after incubation with PF 4 with barbital buffers containing progressively increasing concentrations of sodium chloride (0.15, 0.5, 1.0, or 3.0 M). In a separate series of experiments, sections of kidney cortex were incubated with PF 4, washed with BBS, followed by incubation with sodium heparin (isolated from porcine mucosa, Biosynth, Inc., Chicago, Illinois) at concentrations ranging from 80 μg/ml to 10 mg/ml). The sections were washed with BBS and indirect immunofluorescence procedures were performed as outlined above.

Binding of ^{125}I-PF 4 to isolated glomerular basement membrane (GBM) in vivo

Preparation of GBM. Glomeruli were isolated from kidneys of 30 male Sprague-Dawley rats (Harlan SD, Madison, Wisconsin) by sequential sieving through graded sized mesh screens [13] and purified by osmotic lysis in distilled water, treatment with deoxyribonuclease I (Sigma Chemical Company, St. Louis, Missouri) and sodium deoxycholate according to the method of Meezan, Hjelle, and Brendel [14]. Following extensive washing with PBS, the isolated GBMs were fragmented by brief sonication (3 × 30 sec bursts) on ice using an ultrasonic cell disruptor®, (Model W-220F, Heat Systems Ultrasonics, Inc., Plainview, New York) at an amplitude setting of 6. Protein concentration of GBM suspensions was measured using the method of Lowry et al [15].

Preparation of ^{125}I-PF 4. Iodination of PF 4 was performed by reacting 10 μg of PF 4 with 50 μg chloramine T plus 1 mCI of sodium ^{125}Iodide (Amersham, Arlington Heights, Illinois) [16]. The PF 4 sample was then passed through a heparin sepharose column and free ^{125}I was eliminated by several washes with BBS. Bound ^{125}I-PF 4 was eluted from the column by washing it with 3.0 M NaCl barbital buffer and collecting it in 0.5 ml fractions. Radioactivity of each fraction was assessed using a gamma scintillation counter (Beckman Instruments, Fullerton, California). The two fractions with the highest number of counts were pooled and used fresh or stored at −25°C for subsequent studies. This method produced ^{125}I-PF 4 with an activity of approximately 10,000 cpm/ng (6.0×10^6 cpm/ml). ^{125}I-PF 4 (0.1 ml) was added to unlabeled PF 4 in 0.6 M NaCl to give a final concentration of 500 μg/ml of the platelet protein. Polyethylene glycol (6000 daltons) was added to the solution to make a final concentration of 0.88% to reduce nonspecific adherence of PF 4 to the sides of pipets and test tubes during subsequent dilutions [17]. This stock solution was then diluted to make graded concentrations of PF 4 ranging from 50 to 500 μg/ml.

Protocol. To examine PF 4 binding to isolated GBM in vitro the following experiments were performed:

(1) GBM (15 μg in 100 μl) was added to 800 μl of 0.02 M PBS containing 1% bovine serum albumin followed by the addition of 100 μl of PF 4 dilutions ranging in concentration from 0.5 μg to 100 μg.

(2) To examine an ionic interaction between PF 4 and the GBM, sodium heparin (200 μg) was added to the GBM suspension 10 min following the addition of PF 4. Background counts were assessed by adding the PF 4 dilutions into separate tubes containing buffer without GBM. The tubes were allowed to incubate for 2 hr at room temperature on a tilting mixer, then washed three times by repeated centrifugation at ×3,000*g* and resuspension in buffer. Total counts were determined in separate tubes containing 100 μl of each PF 4 dilution. Relative amounts of PF 4 in each tube were determined by measuring and recording counts per minute and comparing these with total counts in tubes of known PF 4 concentrations. PF 4 bound to GBM was determined by subtracting background counts from counts obtained from tubes in which PF 4 was added to GBM suspensions.

Binding of PF 4 to glomerular structures in vivo. The following studies were performed to assess the potential of purified human PF 4 to bind to kidney polyanion in vivo following intravenous administration into male Sprague-Dawley rats. Rats were lightly anesthetized with ether. Then 0.5 ml of 0.6 M NaCl barbital buffer containing 125 or 250 μg of purified PF 4 was injected into a tail vein. Control rats received an equal volume of 0.6 M NaCl, 0.15 M sodium barbital, pH 7.4, without PF 4. Five minutes following injection of PF 4 or diluent the animals were sacrificed, kidneys were removed, and cortical tissue samples were trimmed and frozen for subsequent indirect immunofluorescence as outlined above.

To further assess an ionic interaction of PF 4 with GPA, rats were lightly anesthetized with ether, then given polyethyleneimine (PEI), 1800 daltons (Arsynco Inc., Carlstadt, New Jersey) prior to administration of PF 4. Since PEI is known to bind to

GPA [2, 3] prior treatment with this synthetic polycation was anticipated to block PF 4 binding. PEI was diluted in 0.05 M PBS, pH 7.2, to make a final solution containing 0.5% PEI with a pH of 7.4. In control experiments 0.05 M PBS without PEI was used. Four rats were given PEI at a dose of 15 μg/g body wt i.v. followed 5 min later by 125 μg of PF 4. An equal number of control rats were given PBS followed by the same dose of PF 4. Five minutes following PF 4 the rats were sacrificed, kidneys were removed, and cortical tissue samples were trimmed and frozen in isopentane pre-cooled in liquid nitrogen. Cortical tissue samples were sectioned and indirect immunofluorescence was performed as outlined above. Similarly, post-treatment with heparin, which has a high affinity for PF 4 [18] may remove PF 4 from GPA. To examine this hypothesis, four rats were given an intravenous injection of 125 μg of PF 4, i.v., followed 5 min later by 500 μg of sodium heparin in 0.5 ml of PBS. Control rats received an equal dose of PF 4 followed by PBS without heparin. Five minutes after the heparin injection or PBS the rats were sacrificed, kidneys were removed, sliced, and frozen. Localization of PF 4 was assessed by immunofluorescence microscopy.

Ultrastructural localization of PF 4 binding sites. PF 4 binding sites in the glomerular capillary wall following intrarenal perfusion were assessed at the ultrastructural level utilizing immunoperoxidase methodology. A total of six rats were anesthetized with sodium pentobarbital, 60 mg/kg i.p. The left femoral artery was gently dissected free from connective tissue and a PE-10 catheter was threaded along the artery up into the abdominal aorta until the tip was localized just below the left renal artery. The catheter was then tied in place with suture. The left renal vein and the aorta above the left renal artery were freed from surrounding connective tissue and all arterial tributaries from the aorta between the catheter and the left renal artery were ligated. Immediately prior to perfusion, the aorta above the left renal artery was clamped and perfusion of oxygenated Krebs-Henseleit saline (KHS) buffer started immediately followed by puncture of the left renal vein. The kidney was washed free of blood by perfusion of 5 ml warm (37°C) KHS over 1 min followed by perfusion of 500 to 600 μg of PF 4 in 3 ml of saline containing 1% BSA ($N = 3$) or diluent alone [0.15 M NaCl, 1% BSA without PF 4 ($N = 3$)]. After pefusion of PF 4 or diluent alone the kidneys were washed with 4 ml of KHS over 3 min followed by perfusion of 2 ml of fixative solution comprised of 3.0% glutaraldehyde, 1.4% paraformaldehyde in cacodylate buffer, pH 7.4. Aldehyde groups were quenched by perfusion of 10 ml 0.1 M Tris-HCl, pH 7.4, immediately after fixation. Kidney cortex was sliced and placed back into Tris-HCl for 4 to 5 days, then several slices were frozen in pre-cooled isopentane and stored at −25°C for later use. Forty to sixty micrometers thick sections were cut from the remaining tissue using a tissue chopper (Smith-Farquhar-Sorvall,® Newton, Connecticut) and incubated in immunoreagents according to the following time schedule: *(1)* 2 hr in normal goat IgG; *(2)* 3 hr in anti-PF 4 serum, normal rabbit serum or anti-PF 4 serum adsorbed with PF 4, and *(3)* 2 hr in horseradish peroxidase conjugated $F(ab')_2$ fragment of goat anti-rabbit IgG (Cappel Laboratories, Westchester, Pennsylvania). Tissue sections were washed three times with PBS (10 min each), between and following all incubation steps. Peroxidase activity was revealed according to the method of Graham and

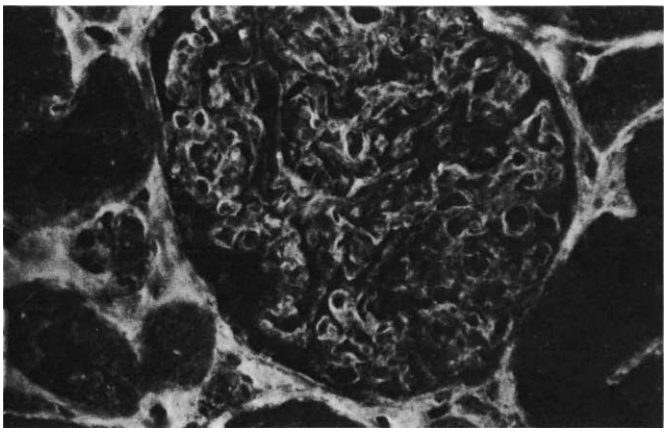

Fig. 1. *Immunofluorescence localization of PF 4 in a section of human renal cortex following incubation with the platelet protein and subsequent washing with barbital buffered saline (BBS).* Localization of PF 4 occurs in glomerular peripheral capillary loops and the mesangium. Also peritubular capillaries and/or tubular basement membrane stain for PF 4. (×250)

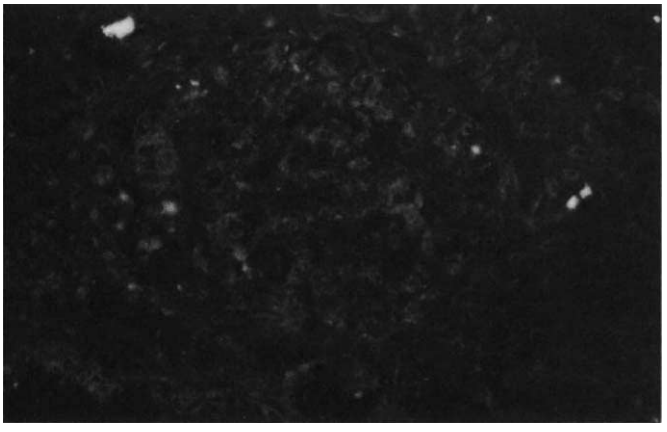

Fig. 2. *Same as Figure 1, except a wash with buffer containing 1.0 M NaCl in place of BBS.* After incubation with PF 4, washing with high molar salt (1.0 and 3.0 M) removed PF 4 from the section as illustrated by minimal background staining. (×250)

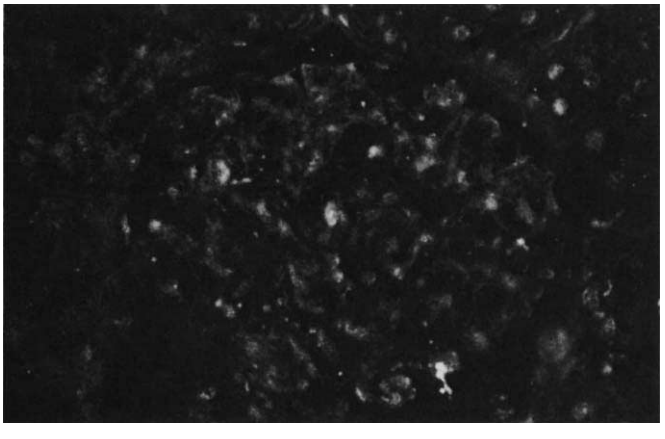

Fig. 3. *Same as Figure 1 except after incubating with PF 4, a subsequent incubation with heparin (10 μg/ml).* PF 4 has been removed from the section as indicated by minimal background staining. (×250)

Karnovsky [19]. The peroxidase reaction was not allowed to proceed beyond 2 min. Sections were washed, osmicated for 30 min with 2.0% osmium tetroxide (OsO_4) then dehydrated and flat-embedded in Epon using standard laboratory techniques. Sliver-gold sections of glomeruli were cut from cross sections of the tissue block, examined, and photographed using an electron microscope (Philips 301). In addition, the indirect immunoperoxidase technique was performed on 6-μm frozen tissue sections for light microscopy in a similar manner as described above except that sections were incubated for 30 min in each immunoreagent and the peroxidase reaction time was allowed to progress for 5 to 15 min.

Results

Localization of PF 4 in human and rat kidney sections incubated with the platelet protein showed similar positive immunofluorescent staining patterns. All sections showed a strong binding of PF 4 to the walls of peripheral capillary loops and to the mesangium (Fig. 1). In addition, peritubular capillaries and/or tubular basement membranes showed strong staining for PF 4 which tended to be more intense in human tissue sections when compared to sections of rat kidney. Since PF 4 was layered onto cut tissue sections, exposed cellular anionic substances were available for PF 4 binding, evidenced by a higher than usual background fluorescence. Sections receiving diluent without PF 4 showed only a minimal background including a slight nuclear staining. Washing the sections with 0.5 M NaCl in barbital buffer following PF 4 enhanced staining of glomerular structures and reduced background staining. Staining was readily removed by washing the sections in barbital buffer containing 1.0 or 3.0 M NaCl (Fig. 2). Treatment of sections with heparin (80 μg/ml to 10 mg/ml) also removed PF 4 as indicated by only faint staining of glomerular and peritubular structures (Fig. 3).

PF 4 binding to isolated GBM. The total amount of PF 4 nonspecifically bound to surfaces of plastic tubes was less than 4% of the total amount of PF 4 bound to GBM. Radiolabelled PF 4 (10 to 100 μg) showed a strong affinity for isolated GBM. A linear relationship between the amount of ^{125}I-PF 4 added to the GBM suspension and the amount bound was shown to occur between 10 and 40 μg of added ^{125}I-PF 4 (Fig. 4). However, this relationship diminished when more than 40 μg of ^{125}I-PF 4 were added to the GBM suspension indicating saturation of PF 4 binding sites. The addition of 200 μg of heparin to the suspension 10 min following PF 4 almost completely abolished PF 4 binding, showing a maximum binding of 4.6% of that observed with GBM plus PF 4, in the absence of heparin (Fig. 4).

Administration of PF 4 in vivo. PF 4 showed a high affinity for GBM and the mesangium as indicated by strong staining of these structures by immunofluorescence following administration of as little as 125 μg of purified PF 4 (Fig. 5). The staining intensity tended to depend on the dose of PF 4 injected and a dose of 250 μg produced more intense immunofluorescent staining of glomerular structures. In addition, peritubular capillaries and tubular basement membranes stained; but staining intensity was much less than that found within glomerular structures (Fig. 5). Control rats and tissue sections receiving diluent without PF 4 or normal non-immune serum in place of primary anti-PF 4 serum showed negligible background staining. Prior administration of PEI blocked PF 4 binding (Fig. 6).

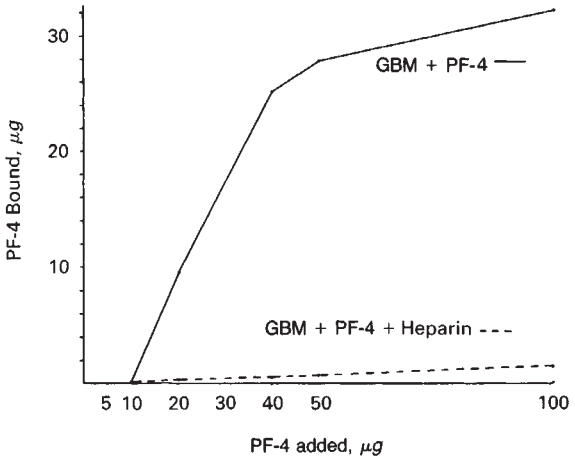

Fig. 4. *Binding of ^{125}I-PF 4 to isolated glomerular basement membrane (GBM).* A linear relationship between the amount of PF 4 added and the amount bound to GBM exists between 10 to 40 μg. This relationship diminishes from 40 to 100 μg. Heparin eliminates most of PF 4 binding to GBM.

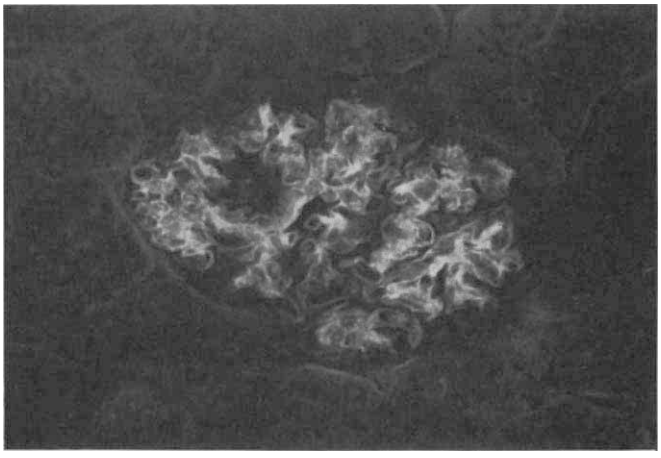

Fig. 5. *Glomerular immunofluorescence localization of human PF 4 after intravenous injection into a rat.* Strong staining of peripheral capillary loops and the mesangium is apparent. (×330)

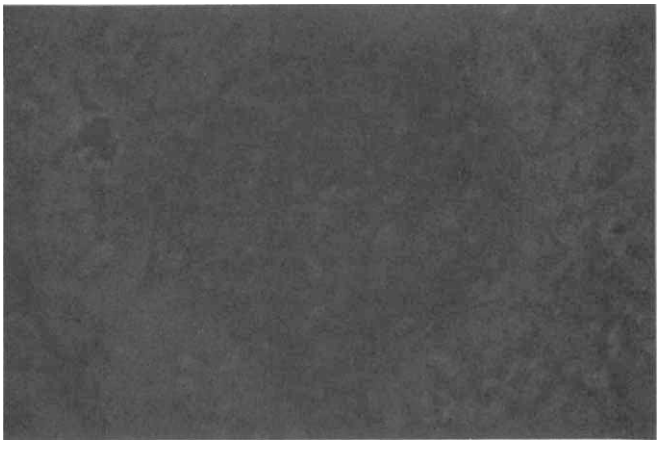

Fig. 6. *Rat glomerulus stained for PF 4 5 min after an injection of polyethyleneimine (PEI) followed by PF 4.* Pre-treatment with PEI blocked PF 4 binding as indicated by the absence of glomerular staining when compared to Figure 5. (×330)

Similarly, heparin administration following injection of PF 4 eliminated staining, indicating removal of the platelet protein from binding sites (not shown).

Light microscopical localization of PF 4 following perfusion revealed intense staining of the GBM in a linear pattern similar to that observed by immunofluorescence following intravenous injection (Fig. 7). In addition, the reaction product was also observed on proximal tubular brush border and within apical vesicles. When non-immune or adsorbed serum was used in place of primary anti-PF 4 serum, control rats and tissue sections were negative (Fig. 8). Similarly, tissue samples from kidneys perfused with diluent alone were negative.

Ultrastructural localization of PF 4 by immunoperoxidase staining revealed that the platelet protein was bound to multiple sites throughout the glomerular capillary wall, particularly within the GBM (Fig. 9). Reaction product was observed throughout the GBM but tended to localize in small punctate sites in both lamina rarae, especially in the lamina rara externa (Fig. 9). Reaction product was also observed on the surfaces of endothelial and epithelial cells, but the strongest staining was observed just beneath epithelial cells adjacent to the GBM. Staining was completely absent in non-immune serum control rats (Fig. 10); however, a very faint staining of glomerular structures was observed in kidneys perfused with diluent without PF 4, possibly reflecting cross reaction with endogenous rat PF 4 released during surgical procedures.

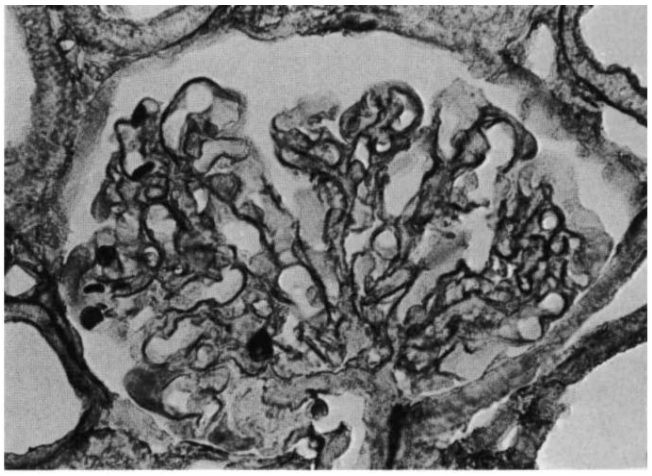

Fig. 7. *Immunoperoxidase localization of human PF 4 following intrarenal perfusion of the platelet protein.* Note the linear staining of glomerular peripheral capillary loops. (×400)

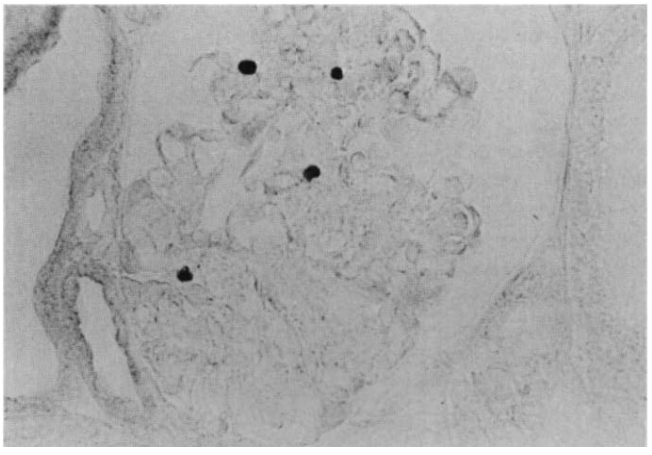

Fig. 8. *Control for tissue section depicted in Figure 7.* The section was treated with non-immune rabbit serum in place of anti-PF 4 serum followed by subsequent peroxidase staining procedures. (×400)

Discussion

The present study allows the following conclusions with regard to the interaction of PF 4 with GPA: *(1)* Purified PF 4 binds avidly to human and rat GBM and epithelial and endothelial cell surfaces; *(2)* PF 4's interaction with glomerular structures is ionic in that binding can be blocked by prior exposure of GPA with a synthetic polycation PEI and can be removed by post-treatment with heparin or high ionic strength buffer; *(3)* PF 4 binding sites in rat GBM, revealed by immunoperoxidase cytochemistry, are heterogeneous in distribution and show a similar pattern as described for glomerular anionic sites stained by cationic dyes.

PF 4 is a platelet-specific protein (31,000 daltons) [20] complexed to a proteoglycan carrier rich in chondroitin sulfate [21] contained in alpha granules. The PF 4-carrier complex readily undergoes dissociation in high ionic strength buffers or through interaction with the glycosaminoglycan heparin [21]. Although PF 4 is not classically considered a "cationic macromolecule" (pI = 7.6), due to its unique distribution of lysine residues, it behaves like a polycation and strongly interacts with negatively charged sulfate groups on glycosaminoglycans [18].

Recent studies have shown that PF 4 can also interact with heparan sulfate on endothelial cell surfaces in culture [22], demonstrating the binding of this platelet protein in a living biological system. Since the GBM is comprised in part of heparan sulfate [23–25] and mesangial and epithelial cells are known to synthesize glycosaminoglycans in culture [26, 27], PF 4 binding to glomerular structures may, at least in part, be attributed to its interaction with glycosaminoglycans. Heparin strongly binds to PF 4 in vitro [18] and has recently been shown to displace PF 4 from heparan sulfate present on endothelial surfaces [22]. Our studies also showed that heparin could remove PF 4 from glomerular binding sites, in vitro and in vivo, supporting such a PF 4-heparan sulfate interaction. Even though PF 4 is known to have a strong affinity for N-sulfated glycosaminoglycans, this interaction is ionic and binding to other glomerular polyanionic constituents such as the sulfated glycoprotein entactin [28] and sialic acid containing glycoproteins such as laminin [29–32] and fibronectin [30, 33–35]; others as yet undefined, cannot be excluded. The ultrastructural immunoperoxidase observations in our study support multiple PF 4 binding sites in that distribution of reaction product was heterogenous (punctate densities in the laminae rarae in addition to diffuse GBM staining and endothelial and epithelial cell coat staining). Our studies do not define which polyanionic component within the GBM comprises the major binding site for PF 4. An answer to this question may await defined studies utilizing enzymatic attack on specific polyanionic moieties followed by isotopic PF 4 binding assays.

The GBM, by virtue of its high polyanionic content, forms a charge barrier to circulating anionic macromolecules [36–40]. A number of recent studies have shown that administration of

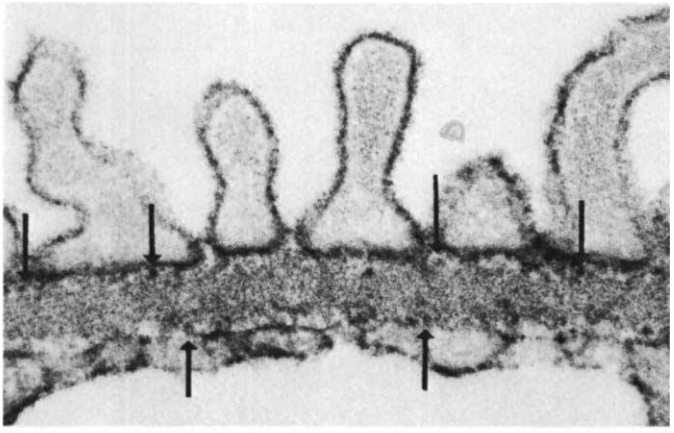

Fig. 9. *Immunoperoxidase cytochemical localization of PF 4 following intrarenal perfusion.* Reaction product is present throughout the GBM but particularly at small punctate sites in the laminae rarae (*arrows*). Also, surfaces of endothelial and epithelial cells stain for PF 4. [Osmium-reaction product (reduced diamino benzidine) ×69,000]

Fig. 10. *Control for Figure 9.* The tissue sample was treated with non-immune rabbit serum in place of primary anti-PF 4 serum. Note the absence of reaction product throughout the glomerular capillary wall. Only osmium staining is apparent resulting in low contrast and detail. (×69,000)

synthetic polycations in vivo can neutralize GPA and lead to alterations in permeability to circulating macromolecules [1, 4–6]. It is also of considerable interest that removal of heparan sulfate by enzyme digestion with heparitinase increases glomerular permeability to the tracer macromolecule ferritin [40]. Neutralization of GPA and the resultant perturbation of glomerular permeability is of particular importance since endogenous polycations may be released from inflammatory cells and/or platelets during glomerular disease processes; these may bind to GPA and induce permeability defects within the GBM.

Previous studies in our laboratory have shown that a synthetic polycation, PEI can increase glomerular permeability to passively administered antigen and antibody and lead to enhanced deposition and altered distribution of immune complexes [7]. Endogenous cationic proteins once released may behave in a similar fashion at a local level and affect the GBM. This concept has been supported by Camussi et al [41] in a recent study describing GBM localization of neutrophilic cationic proteins associated with loss of GPA in lupus nephritis. Platelets have been implicated as mediators in glomerulonephritis [8–11] supporting such a role for PF 4 and other platelet polycationic proteins as mediators in immune complex deposition. Also, blood levels of PF 4 are known to be markedly elevated during glomerulonephritis [11].

PF 4 is released from platelets as a high molecular weight proteoglycan-PF 4 complex. The degree of dissociation of PF 4 from its carrier following release is unknown. PF 4 has a much higher affinity for heparan sulfate than chondroitin sulfate [18, 21]. Thus, it is likely that transfer of PF 4 from its chondroitin sulfate-rich carrier to heparan sulfate or other GPA within the glomerular capillary wall may occur during glomerular disease. Intraglomerular activation of platelets would be expected to result in high concentrations of PF 4 in the milieu directly adjacent to and accessible to the GBM. Our studies do not address the above issues, but they do indicate that dissociated, purified PF 4 is capable of avid binding to GPA. That free PF 4 binds to GPA in sufficient quantities to perturb glomerular permeability during glomerular disease processes remains to be determined. It is important to point out that a myriad of other cationic proteins may also be released from platelets and leukocytes during glomerulonephritis and collectively may influence glomerular permeability. Such platelet-GBM interactions and permeability perturbations would occur at a local level and experimental reproduction of this phenomenon may require intravenous administration of large quantities of platelet proteins. Studies are currently underway in our laboratory to examine the potential of PF 4 to induce glomerular permeability defects and its role in glomerular disease processes.

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